

SOME ANATOMICAL EFFECTS OF KINETIN AND RED LIGHT ON DISKS OF BEAN LEAVES¹

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The application of kinetin (6-furfurylamino-purine) produces some effects similar to those induced by exposure to red light (Miller, 3, and Scott and Liverman, 5). Both cause increased expansion of disks from bean leaves, greater elongation in stems and petioles of bean seedlings, and increased germination of lettuce seeds in the dark. Both treatments inhibit internodal elongation in excised peas. Because of the striking resemblance in these effects, Miller (3) suggested that these two treatments may operate through the same biological mechanism. A subsequent study of the germination of lettuce seeds in the dark indicated that kinetin is not involved directly in the photoreaction or in the reactions which immediately precede or follow it (Miller, 4). Scott and Liverman (5) also doubted if kinetin controlled the same reaction step as red light. The objective of the present investigation was to determine if anatomical differences occur between samples of disks from bean leaves treated with kinetin and those exposed to red light.

MATERIALS AND METHODS

Disks, 7.0 mm in diameter, were cut from the first foliage leaves of 8-day-old bean seedlings, *Phaseolus vulgaris* L. (Burpee stringless green pod), which had been grown in the dark. Four groups of ten disks each were placed in Petri dishes on Whatman's #3 filter paper, and 7.0 ml of nutrient solution prepared according to a formula described by Miller (3) was added. One group of disks served as a control; two groups had different concentrations of kinetin added to the nutrient solution; the fourth was exposed to red light for 12 minutes. Red light was filtered from two 15 watt fluorescent bulbs through two sheets of Du Pont 300 MCS red cellophane. The light source was placed 30 cm from the leaf disks. Manipulations were made under a Wratten Series 3 (green) safe-light, and temperature was maintained at 25° C. At the end of 48 hours, the increase in the size of the diameters of the disks was measured with the aid of an eyepiece micrometer in a dissecting microscope. An analysis of variance was made and Duncan's multiple range test (1) was applied.

The second experiment was undertaken after the results of the first were known; the same methods were employed in both experiments, but the leaf disks were cut 5.0 mm in diameter. One group served as the control. Two groups were exposed to red light; the first for 5 minutes, and the other for 10 minutes. Kinetin was added to the nutrient medium of the fourth group. The fifth group of disks was supplied with kinetin and also exposed to 5 minutes of red light. At the end of 48 hours all disks were measured and prepared for histological study.

All disks were killed and fixed in Randolph's solution, dehydrated through a tertiary butyl alcohol series, and embedded in paraffin. Sections were cut 8 μ thick with a rotary microtome and stained with tannic acid-ferric chloride and safranin. The width of the palisade cells was measured with an eyepiece micrometer.

RESULTS

Disks treated with kinetin and those exposed to 12 minutes of red light were larger in diameter after 48 hours than those of the control (table I). The difference in effect of the two concentrations of kinetin on disk size was significant at the 5 % level.

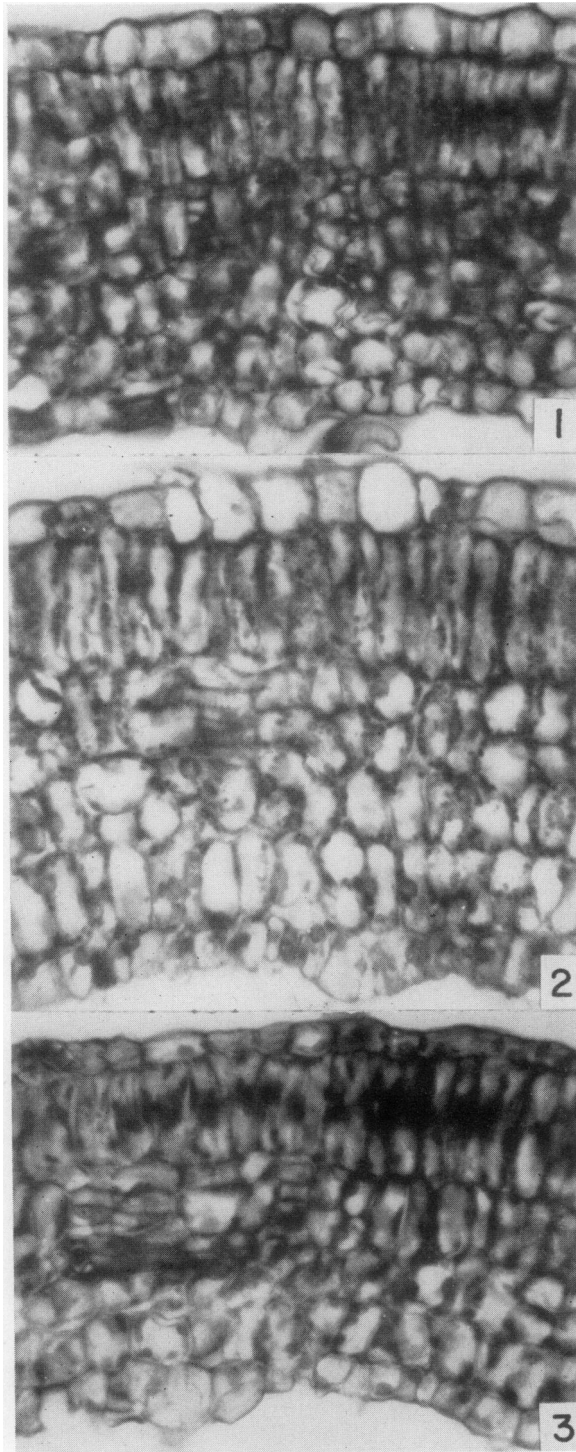
Table I shows a comparison of the average width of the palisade cells in disks at the conclusion of the first experiment. Palisade cells are wider in both of the kinetin-treated samples than in either the control or those irradiated with red light. The greater increase in width of the palisade cells from treated leaf disks is evident in the photographs. Width of the palisade cells in the disks of the control (fig 1) and in those exposed to red light (fig 3) did not differ significantly. Palisade cells in the kinetin-treated disks (fig 2) were wider, and the disks were thicker,

TABLE I
GROWTH OF DISKS AND WIDTH OF
PALISADE CELLS FROM BEAN LEAVES

TREATMENT	GROWTH OF DISKS (MM)	WIDTH OF PALISADE CELL (μ)
None	1.42 \pm 0.04*	6.53 \pm 0.11*
2.0x10 ⁻⁴ M Kinetin	2.53 \pm 0.05	8.94 \pm 0.22
0.4x10 ⁻⁴ M Kinetin	2.22 \pm 0.15	8.69 \pm 0.28
12 Min red light	2.39 \pm 0.08	6.94 \pm 0.19

* Standard error

¹ Received August 7, 1959.



FIGS. 1-3. Cross section of disks from bean leaves 48 hours after placing in nutrient solution (480 X). Figure 1, control; figure 2, 2×10^{-4} M kinetin added to nutrient solution. Figure 3, 12 minutes red light given at time of placing in nutrient solution.

indicating that cells enlarged more in all directions. Neither of the treatments produced any observable changes other than size.

The second experiment also indicates that disk enlargement is increased by either kinetin or exposure to red light (table II). Growth promotion by kinetin was significant at the 5 % level in this experiment. Smaller disks were used in the second experiment than in the first. The darkroom was considered adequate at the time of the first experiment. However, since Miller (4) found that in the germination of lettuce seed the effectiveness of kinetin was increased by small amounts of light, some improvements were made in the darkroom before the second experiment. It may be that a decreased exposure to light in the second experiment reduced the kinetin effect. The disks grown in a solution of kinetin and exposed to red light were slightly larger (significant at the 5 % level) than disks receiving only one of the treatments.

Results in the second experiment were in agreement with those of the first in respect to cell enlargement (table II). Moreover, adding kinetin to the nutrient solution plus exposure to red light resulted in greater increase in cell width (significant at the 1 % level) than either treatment alone.

TABLE II
GROWTH OF DISKS AND WIDTH OF
PALISADE CELLS FROM BEAN LEAVES

MIN RED LIGHT	M CONC KINETIN	DISK GROWTH (MM)	PALISADE CELL WIDTH (μ)
0	0	$0.40 \pm 0.07^*$	$6.16 \pm 0.20^*$
5	0	1.30 ± 0.08	6.44 ± 0.08
10	0	1.19 ± 0.12	5.80 ± 0.12
0	10^{-4}	0.88 ± 0.12	7.22 ± 0.12
5	10^{-4}	1.58 ± 0.16	8.62 ± 0.24

* Standard error

DISCUSSION

This study is in agreement with the previously reported finding that either kinetin treatment or exposure to red light produces increased expansion of disks of bean leaves. Miller (3) and Skoog and Miller (6) attributed the kinetin effect to greater cell enlargement, but published no data. Our results substantiate their explanation and demonstrate the extent of the increased cell enlargement. In addition, this study shows that cells from disks treated with red light were no larger than the control. The larger size of the disks after red light treatment must be due to an increase in the number of cell divisions.

This investigation was initiated to determine if kinetin and red light influenced growth through the same or a similar mechanism. If kinetin merely substituted for red light, the growth pattern resulting from either treatment would be the same. Our re-

sults show that kinetin stimulated growth by increased cell enlargement, but that red light stimulated growth by an increased rate of cell division. As stated earlier other investigations (4,5) have given some evidence that kinetin and red light are probably not involved in the same reaction. Our results further substantiate this concept and show conclusively that kinetin does not merely substitute for red light.

Although the types of growth resulting from kinetin and red light are different, an interaction between kinetin and red light may exist. Disks treated with both red light and kinetin were larger than if given either treatment alone. If kinetin stimulates greater cell enlargement and red light increases the rate of cell division, this result would be expected. With this simple relationship the cells should be the same size with both treatments or with kinetin alone. However, the cells from disks receiving both red light and kinetin were significantly larger ($P < 0.01$) than those from disks treated with kinetin alone. These results indicate that there is an interaction between kinetin and red light. Further research is needed to determine the nature of such an interaction. The results presented here indicate that the answer may be found in the complicated relationship between cell enlargement and cell division.

SUMMARY

I. Red light increased expansion of disks cut from etiolated bean leaves, but did not significantly alter the size of palisade cells.

II. Kinetin increased the expansion of disks cut from etiolated bean leaves and also caused a marked increase in cell size.

III. The action of kinetin and red light together caused a greater increase in size of disks cut from etiolated bean leaves than either treatment alone.

IV. Kinetin did not physiologically replace or substitute for red light.

V. An interaction between the effects of kinetin and red light probably exists, but its nature has not been determined.

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